CONCISE COMMUNICATION

Haemophilus segnis: a rare cause of endocarditis

C. J. Somers[1,2,], B. C. Millar[3], J. Xu[3], D. P. Moore[2], A. M. Moran[2], C. Maloney[2], B. Keogh[2], P. G. Murphy[2] and J. E. Moore[3]*

[1]Department of Microbiology, The Laboratories, The Adelaide and Meath Hospital, Dublin 24, [2]Adelaide and Meath Hospital incorporating The National Children's Hospital, Tallaght, Ireland and [3]Molecular Epidemiology Research Unit, Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast, Northern Ireland, UK

[*]Fax: +353 14 143980 E-mail: csomers@rcsi.ie

This report presents a case of endocarditis due to *Haemophilus segnis*, which represents a speciation difficulty for the routine laboratory. In this study, a molecular approach provided speciation, which was confirmed phenotypically by a reference laboratory. The use of molecular genotypic analysis is an additional strategy in the investigation of endocarditis. It has applications not only in isolate identification but also in primary detection of infection, particularly in patients whose blood is culture negative by conventional methodologies.

Keywords *Haemophilus segnis*, endocarditis, molecular diagnosis, genotypic speciation

Clin Microbiol Infect 2003; 9: 1048–1050

Infective endocarditis can still present a diagnostic dilemma, particularly when fastidious microorganisms are involved. The introduction of molecular biological techniques can aid the diagnosis of such presentations. We report on the case of a 59-year-old-male who presented with a history of slurred speech of 20 min duration. This followed a 6-week history of intermittent headache, sweating, and moderate anorexia. The patient described a history of bleeding gums, but no recent dental procedures. On examination, he was coherent, afebrile, and his vital signs were normal. No neurologic deficits were detected. A grade 3 systolic murmur was audible, and there were no added cardiac sounds. Rashes or other skin lesions were not observed. Laboratory investigations revealed: a white cell count of 9.2×10^9/L, with 75% neutrophils; hemoglobin, 12.4 g/dL; platelets, 276×10^9/L; urea, 5.6 mmol/L; creatine, 100 μmol/L; C-reactive protein, 101 mg/L; alanine aminotransferase (ALT), 43 U/L; and γ-glutamyl transaminase, 68 IU/L. Chest X-ray, CT brain scan, carotid Doppler, ultrasound, spiral CT of the abdomen and isotope bone scan were normal. ECG and Holter studies showed normal sinus rhythm. Transesophageal echocardiography (TOE) showed moderate mitral regurgitation and evidence of two ruptured chordae. Interpretation of the TOE tentatively suggested the presence of vegetations. Early-morning urine samples (×3) were negative for *Mycobacterium tuberculosis*. Serology was negative for *Chlamydia*, *Mycoplasma*, *Coxiella*, *Toxoplasma*, *Brucella*, and *Leptospira*.

Five blood culture sets, including aerobic and anaerobic bottles, were taken. Growth (BacT/Alert, Organon Teknika Corp., Durham, NC, USA) was detected in aerobic bottles inoculated on days 9 and 16 post-admission. Cultures were flagged positive after 4 days and 39 h of incubation, respectively. Subculture was performed on chocolate agar at 37 °C with 5% (v/v) CO_2. The isolates were identified as a *Haemophilus* sp., using API NH (BioMerieux, Marcy l'Etoile, France), which gave a 57.5% matching probability against *H. aphrophilus*. The isolates were sent for further identification to a reference laboratory (Laboratory of Hospital Infection, Public Health Laboratory Service, Colindale, UK), where they were identified phenotypically as *H. segnis*, based on their reactions to a panel of substrates, and on growth characteristics used to differentiate *Haemophilus* spp. [1]. These isolates were sensitive to amoxicillin, cefotaxime and ciprofloxacin, and resistant to clarithromycin, as

determined by a disk diffusion assay using a modified Stokes procedure [2].

On day 18 post-admission, treatment was commenced with intravenous ceftriaxone (1 g once daily) and intravenous gentamicin (280 mg once daily). C-reactive protein declined over the next 3 days to 28 mg/L, with a corresponding clinical improvement. The antibiotic regimen was changed to oral amoxicillin, 1 g every 8 h and ciprofloxacin, 500 mg twice daily, at discharge 26 days post-admission, and the patient continued on this treatment for a further 5 weeks. He remained clinically well, with a C-reactive protein level of 3 mg/L 5 months post-discharge.

Retrospectively, the *Haemophilus* sp. isolates were further examined for identification purposes by PCR amplification and sequence analysis of a partial region of the 16S rRNA gene (216 bp), as previously described [3]. The sequence obtained (GenBank Accession No. AF409116) was compared with those stored in the GenBank nucleotide database using BLAST alignment software, and this indicated that the isolate was most closely related to four species (173/183 bp, 94.5% homology), namely, *H. paraphrophilus* (M75042), *H. aphrophilus* (M75041), *H. parainfluenzae* (M75040) and *H. segnis* (AF224299). In order to differentiate between these species, a larger region (605 bp) of the 16S rRNA gene was examined, employing the primer pair PSL [4], P13P [3]. The resulting sequence (552 bp, bankit470272) was 99.5% homologous with *H. segnis* (AF224299), and was distinct from *H. paraphrophilus* (M75042), *H. aphrophilus* (M75041), and *H. parainfluenzae* (M75040), as these aligned with the larger sequence to give 98.7%, 98% and 98% homology, respectively.

The HACEK group of organisms includes *Haemophilus* spp. (*H. parainfluenzae*, *H. influenzae*, *H. aphrophilus*, *H. paraphrophilus*), *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella kingae*. This grouping does not represent a separate bacterial taxonomic class, and it is not monophyletic, based on the 16S rRNA sequence. Members of the HACEK group of organisms do, however, share common physiologic features, including growth enhancement by CO_2, slow growth rate (may require a period of 2 weeks to be isolated from blood cultures), and colonization of the oropharynx. The most serious disease manifestation of the HACEK microorganisms is endocarditis, where their incidence has been reported to be approximately 3% [5]. For a comprehensive review of this group of organisms causing endocarditis, see Das et al. [6]. The clinical presentation is principally as a chronic endocarditis, but acute presentations do occur [7].

The genus *Haemophilus* comprises 15 species. *H. segnis* is a rarely recognized commensal in the oropharynx, first isolated from human dental plaque material [8]. There have been relatively few documented reports on its pathogenic involvement in infection. This may be because of the phenotypic resemblance of *H. segnis* to *H. parainfluenzae*. Both organisms are V-factor growth dependent, have variable catalase activity, and are ortho-nitrophenyl-D-galactoside (ONPG) variable [1]. They also share negative indole and lactose activities. To our knowledge, this is the second reported case of *H. segnis*-related endocarditis (see Carson et al. [9]), and the first diagnosed using PCR. The other cited case was of a 76-year-old woman who recovered following ampicillin therapy for 57 days combined with a 10-day course of netilmicin [9]. *H. segnis* has occasionally been reported as a causal agent of infection in cholecystitis [10], periodontitis [11], appendicitis [12,13], and septicemia [14].

Medical treatment, either alone or associated with valve surgery, is reported to cure 82–87% of patients with endocarditis caused by the HACEK group organisms [15]. In the past, ampicillin plus gentamicin was the treatment of choice. The increasing prevalence of β-lactamase-producing organisms has necessitated a change in treatment strategy to include a β-lactamase-stable cephalosporin in place of ampicillin [16]. The present isolate was sensitive to amoxicillin, making the use of a cephalosporin unnecessary. The patient was treated for 1 week with intravenous ceftriaxone and gentamicin, and for a further 5 weeks with oral amoxicillin and ciprofloxacin. As the patient was improving on the oral regimen, which had proven in vitro activity, this was continued on an outpatient basis, with a good outcome. There are insufficient published data on the treatment of *H. segnis* endocarditis to allow valid consensus guidelines [17].

REFERENCES

1. Holt JG, Krieg NR, Sneath PHA, Bergy D, eds. *Manual of determinative bacteriology*, 9th edn. Lipincott: Williams & Wilkins, 1994: 1. 286.
2. Anonymous. A guide to sensitivity testing. Guidelines by the Working Party of the British Society for

Antimicrobial Chemotherapy BSAC. *J Antimicrob Chemother* 1991; 27(suppl D): 1–50.
3. Millar B, Moore J, Mallon P *et al.* Molecular diagnosis of infective endocarditis—a new Duke's criterion. *Scand J Infect Dis* 2001; 33: 673–80.
4. Campbell PW, Phillips JA, Heidecker GJ, Krishnamani MR, Zahorchak R, Stull TL. Detection of *Pseudomonas* (*Burkholderia*) *cepacia* using PCR. *Pediatr Pulmonol* 1995; 20: 44–9.
5. Steckelberg JM, Melton LJ, Ilstrup DM, Rouse MS, Wilson WR. Influence of referral bias on the apparent clinical spectrum of infective endocarditis. *Am J Med* 1990; 88: 582–8.
6. Das I, DeGiovanni JV, Gray J. Endocarditis caused by *Haemophilus parainfluenzae* identified by 16S ribosomal RNA sequencing. *J Clin Pathol* 1997; 50: 72–4.
7. Brouqui P, Raoult D. Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* 2001; 14: 178–96.
8. Kilian M. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *J Gen Microbiol* 1976; 93: 9–62.
9. Bangsborg JM, Tvede M, Skinhoj P. *Haemophilus segnis* endocarditis. *J Infect* 1988; 16: 81–5.
10. Carson HJ, Rezmer S, Belli J. *Haemophilus segnis* cholecystitis: a case report and literature review. *J Infect* 1997; 35: 85–6.
11. Petsios A, Nakou M, Manti F. Microflora in adult periodontitis. *J Periodont Res* 1995; 30: 325–31.
12. Namnyak SS, Martin DH, Ferguson JD, Chiquito PE, Hughes DF. *Haemophilus segnis* appendicitis. *J Infect* 1991; 23: 339–41.
13. Welch WD, Southern PM Jr, Schneider NR. Five cases of *Haemophilus segnis* appendicitis. *J Clin Microbiol* 1986; 24: 851–2.
14. Bullock DW, Devitt PG. Pancreatic abscess and septicaemia caused by *Haemophilus segnis*. *J Infect* 1981; 3: 82–5.
15. Berbari EF, Cockerill FR, Steckleberg JM. Infective endocarditis due to unusual or fastidious microorganisms. *Mayo Clin Proc* 1997; 72: 532–42.
16. Greene JN, Sandin RL, Villanueva L, Sinnott JT. *Haemophilus parainfluenzae* endocarditis in a patient with mitral valve prolapse. *Ann Clin Lab Sci* 1993; 23: 203–6.
17. Wilson WR, Karchmer AW, Dajani AS *et al.* Antibiotic treatment of adults with infective endocarditis due to streptococci, enterococci, staphylocci, and HACEK microorganisms. *JAMA* 1995; 274: 1706–13.